

FINAL REPORT

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INSTITUTION: The University of Chicago

GRANT TITLE: Characterization of Light and Nitrogen Regulated Gene Expression Pathways in Marine Diatoms

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OBJECTIVE: To define pathways for environmental regulation of gene expression in marine diatoms. Elucidate the extent of light and nitrogen dependent transcriptional and translational control on the expression of nitrate reductase (NR), glutamine synthetase (GS) and the fucoxanthin-chlorophyll a/c pigment protein (FCP); these gene products determine the nitrogen assimilation and light-harvesting capacity of marine diatoms. Define the genomic organization and characterize the 5'-promoter regions for NR, GS and FCP in order to identify sequence motifs which may function as light or nitrogen responsive regulatory elements.

APPROACH: Develop nucleic acid and antibody probes for NR, GS and FCP. Apply these probes in conjunction with activity assays to determine changes in transcript and protein abundance in diatom cultures during shifts in light and/or nitrogen availability to reveal the extent to which transcriptional, translational or post-translational processes control the expression of NR, GS and FCP. Develop universal primers for use in the polymerase chain reaction to isolate DNA clones for NR, GS, FCP and ACTIN. Use these clones to determine the cellular location of these genes and for isolation of genomic clones for characterization of promoter regions for light/nitrogen regulated and constitutively expressed genes respectively.

ACCOMPLISHMENTS: Purification of the NR protein from the diatom Skeletonema costatum was completed and rabbit polyclonal antiserum produced and affinity purified. The affinity purified antiserum is specific and of high titer enabling us to detect NR in protein extracts from as few as 10^4 cells. The crude and affinity purified antisera do not cross-react with green algal or plant NR, consistent with the low sequence homology observed in our NR cDNA clones. Western blot analysis with the NR and FCP antisera revealed a differential light regulation for these two proteins, with the cellular content of NR being up-regulated and that of FCP down-regulated when S. costatum cells were exposed to increased irradiance levels. This observation provides the framework for our continuing studies delineating the pathways by which the same signal (light) can have disparate affects on gene expression.

Purification of the GS protein using ion-exchange chromatography has revealed the presence of at least two isoforms in S. costatum and the kelp Macrocystis pyrifera. These purifications were scaled up to obtain enough antigen for antisera production. Experiments are being conducted to examine the change in abundance of these two isoforms (presumably chloroplastic and cytosolic) in response to ammonium and nitrate enrichment.

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Oligonucleotide primers were designed in the coding and noncoding sequence orientation corresponding to conserved amino acid domains identified in multiple alignments of known eukaryotic NR proteins. Sequence analysis of NR clones obtained by PCR amplification of total DNA and first strand cDNA from S. costatum indicates that the diatom form of NR exhibits only limited sequence identity (ca 30%) with higher plant or fungal forms. Control amplifications of DNA from the marine angiosperm Zostera marina yield fragments which exhibit a sequence identity (46%) comparable to that found among other plant groups. The Z. marina NR clone does not exhibit any specific hybridization on Southern blots with S. costatum DNA supporting the low sequence similarity of plant and diatom NR. This sequence divergence in diatom NRs was also evidenced by amino acid sequences determined for N-terminal and tryptic fragments of S. costatum NR protein, where BLAST searches of the Genbank or SwissProt databases yielded no significant alignments.

A similar approach was utilized for the isolation of GS coding sequences. GS clones from S. costatum and the green alga Dunalliella tertiolecta exhibited high sequence similarity (>60%) to GSII isoforms found in higher plants. Clones for FCP, ACTIN and the small subunit rRNA have also been obtained and are being characterized. FCP, NR, and GS sequences tend to be A+T rich (> 55%) compared to plant or fungal genes. Southern analysis reveals that FCP, NR and GS genes are nuclear encoded. Actin and rRNA clones will serve as control probes for constitutively expressed nuclear genes and are being assessed for use as markers of cellular growth rate during the environmental shifts. The NR clones are being used as templates for hybrid selection experiments and the availability of the NR antisera will enable us to independently confirm the identity of the S. costatum NR clones by immunoprecipitation of the translation products of the selected RNA.

CONCLUSIONS: The research supported by this funding has enabled the development of several molecular probes to critical biosynthetic (NR, GS) and structural proteins (FCP, actin) in marine diatoms. This success will lead to their future application for sensitive monitoring of the molecular events regulating the production dynamics of this critical group of marine algae. Additionally this research has revealed substantial genetic divergence in essential biosynthetic genes which emphasizes that diatoms and other chromophyte algae are not simply brown-plants but represent discrete genetic lineages potentially harboring a wealth of genetic and metabolic innovation that can only be revealed through continued molecular studies of these cells.

SIGNIFICANCE: This comparative approach to the study of environmental control of gene expression will provide new and critical information on the structure of light and nitrogen responsive transcriptional control elements in marine algae. It also represents an initial attempt to delineate signal transduction pathways in diatoms, information which is necessary for understanding environmental regulation of diatom growth in biofilms as well as for our utilization of marine algae in biotechnological applications.

PUBLICATIONS AND ABSTRACTS:

1. Gao, Y., G.J. Smith and R.S. Alberte (1992) Light regulation of nitrate reductase in Ulva fenestrata I. Influence of light regimes on nitrate reductase activity. Mar. Biol. 112:691-696.
2. Smith, G.J., R.C. Zimmerman and R.S. Alberte (1992) Molecular and physiological responses of diatoms to variable levels of irradiance and nitrogen availability: Growth of Skeletonema costatum in simulated upwelling conditions. Limnol. Oceanogr. 37: 989-1007.
3. Gao, Y., G.J. Smith and R.S. Alberte (1993) Nitrate reductase from the marine diatom Skeletonema costatum Biochemical and immunological characterization. Plant Physiol. 103: 1437-1445.
4. Gao, Y., G.J. Smith and R.S. Alberte (1991) Light and circadian regulation of nitrate reductase in Ulva fenestrata (Chlorophyceae). J. Phycol. 27 (Suppl.): 122. Abstract presented at the 4th Int. Phycological Congress.
5. Robertson, D.L., G.J. Smith and R.S. Alberte. (1991) Characterization of glutamine synthetase in two chromophytic algae. J. Phycol. 27 (Suppl.): 520. Abstract presented at the 4th Int. Phycological Congress.
6. Smith, G.J. and R.S. Alberte (1992) Molecular probes of nitrate assimilation in marine phytoplankton. Abstract presented at the ASLO Aquatic Sciences Meeting, Santa Fe, NM. February 1992.

7. DISSERTATIONS SUPPORTED

- Gao, Y. (1997) Nitrate Assimilation: Biochemical and environmental control. Ph.D. Dissertation, Dept of Biological Sciences / Alan Hancock Foundation, University of Southern California, Los Angeles, CA. 200pp.
- Robertson, D.L. (1997) Characterization of glutamine synthetase from the marine diatom Skeletonema costatum. Ph.D. Dissertation. Dept. Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL. 177pp.

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13. ABSTRACT (Maximum 200 words) This report summarizes the results of ONR funded research focused on characterization of the genes and gene products for several biosynthetic and structural proteins found in marine diatoms with the goal to utilize molecular probes generated from these studies to monitor molecular events regulating the production dynamics of marine phytoplankton. The diatom <u>Skeletonema costatum</u> , an ubiquitous coastal species was selected as a model system. The polymerase chain reaction utilizing consensus oligonucleotide primers to conserved amino acid domains was employed was used to isolate genomic and cDNA fragments corresponding to the sequences encoding the nitrogen assimilatory enzymes nitrate reductase (NR) and glutamine synthetase (GS) and the structural proteins actin and the fucoxanthin chlorophyll a/c protein (FCP). NR and GS proteins were purified using affinity, ion-exchange and size exclusion chromatographic techniques. Polyclonal antibodies were generated to these proteins. While enzymatic assays revealed conservation of reaction kinetic mechanisms, molecular and immunological analyses revealed substantial divergence in the primary structure and patterns of environmentally responsive gene expression for these proteins compared to their vascular plant or fungal counterparts. These studies have revealed that chromophyte algae offer a wealth of metabolic innovation not previously described.				
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